

40. Moon, R. C. & Itri, L. M., in *The Retinoids* (eds. Sporn, M. B., Roberts, A. B., Goodman, D. S.) 327-371 (Academic Press, N.Y., 1984).
41. Munoz, A., Zenke, M., Gehring, U., Sap, J., Beug, H., and Vennstrom, B., *EMBO J.* 7, 155-159 (1988).
42. Proudfoot, N. J. & Brownlee, G. G., *Nature* 263:211-214 (1976).
43. Sanger, F., Nicklen, S. & Coulson, A. R., *Proc. Natl. Acad. Sci., USA* 74:5463-5467 (1977).
44. Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., Beug, H. & Vennstrom, G., *Nature* 324:635-640 (1986).
45. Schwartz, H. L., in *Molecular Basis of Thyroid Hormone Action*, J. H. Oppenheimer and H. H. Samuels, Eds, pp. 413-444 Academic Press, N.Y., (1983).
46. Segraves, W., thesis, Stanford University, (1988).
47. Shroder, E., Rapaport, E., Kabeneil, K. & Black, P. H., *Proc. Natl. Acad. Sci., USA* 79:1549-1552 (1982).
48. Sigler, P. B., *Nature* 333: 210-212 (1988).
49. Southern, E. M., *J. Molec. Biol.* 98:503-517 (1975).
50. Sporn, M. & Roberts, A. B., *Cancer Res.* 43:3034-3040 (1983).
51. Sporn, M. B. & Roberts, A. B., in *The Retinoids*, Vol. 1 (eds. Sporn, M. B., Roberts, A. B., Goodman, D. S.) 235-279 (Academic Press, N.Y., 1984).
52. Strahle, U., Klock, G., and Schutz, G. *Proc. Natl. Acad. Sci., USA* 84:7871-7875 (1987).
53. Strickland, S. & Mahdavi, V., *Cell* 15:393-403 (1978).
54. Staden, R., *Nucleic Acid Res.* 10:2951-2961 (1982).
55. Tora, L., Gronemeyer, H., Turcotte, B., Gaub, M-P., and Chambon, P., *Nature* 333:185-188 (1988).
56. Thompson, C. C., Weinberger, C., Lebo, R. & Evans, R. M., *Science* 237:1610-1614 (1987).
57. Tickle, C., Lee, J. & Eichele, G., *Devel. Biol.* 109:82-95 (1985).
58. Umesono, K., Giguere, V., Glass, C., Rosenfeld, M., & Evans, R., *Nature*, 336:262-265 (1988).
59. Wang, S.-Y., LaRosa, G. & Gudas, L. J., *Dev. Biol.* 107:75-86 (1985).
60. Webster, N., Green, S., Jin, J. R., and Chambon, P., *Cell* 54:199-207 (1988).
61. Weinberger, C., Hollenberg, S. M., Rosenfeld, M. G. & Evans, R. M., *Nature* 318:670-672 (1985).
62. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J. & Evans, R. M., *Nature* 324:641-646 (1986).
63. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J., Evans, R. M., *Nature* 324, 641-646 (1986).
64. Wigler, M., et al., *Cell* 16:777-785 (1979).
65. Wolback, S. B. & Howe, P. R., *J. Exp. Med.* 55 62:753-777 (1925).

SPECIFICATION SUMMARY

From the foregoing description, one of ordinary skill in the art can understand that the present invention provides substantially pure DNA which encodes the retinoid receptor protein referred to as retinoic acid receptor protein. The invention also provides a plasmid containing retinoic acid receptor DNA. This plasmid, pHRAR1, has been deposited with the American Type Culture Collection for patent purposes.

The invention is also comprised of retinoic acid receptor proteins, including modified functional forms

thereof, expressed from the DNA (or mRNA) of the invention.

In addition to novel retinoic acid receptor DNA, RNA and protein compositions, the present invention includes chimeric hybrid receptors made by exchanging (1) the N-terminal domains, (2) the DNA-binding domains, and (3) the ligand-binding domains from hGR, hMR, hERR1, hERR2, T₃R_α, T₃R_β, RAR_α, and RAR_β receptors with one another. The chimeric receptors so constructed have DNA-binding domain and ligand-binding domain characteristics similar to the DNA-binding domain and ligand-binding domain characteristics of the respective "parental" receptors from which they originated.

Finally, the present invention involves a bioassay for determining the functional ligands for receptor proteins, both wild-type and chimeric.

The pHRAR1 DNA of the invention can be used to make the retinoic acid receptor proteins, and functional modified forms thereof, in quantities that were not previously possible. The same is true of the chimeric receptors. With the quantities of receptor protein available as a result of the present invention, detailed studies can be made of both the ligand/receptor complexes and the ligand/receptor/HRE complexes. In addition, an adequate supply of the retinoic acid receptor proteins means that they can now be used to screen compounds for retinoic acid receptor-agonists or retinoic acid receptor-antagonist activity. Availability of the receptor proteins also means that they can be used in diagnostic assays to determine the levels of retinoic acid present in various tissues and body fluids.

Without departing from the spirit and scope of this invention, one of ordinary skill can make various changes and modifications to the invention to adapt it to various usages and conditions. As such, these changes and modifications are properly, equitably, and intended to be, within the full range of equivalence of the following claims.

What is claimed is:

1. Substantially pure DNA encoding retinoic acid receptor wherein said retinoic acid receptor is structurally and functionally related to the steroid and thyroid hormone receptors.
2. Substantially pure DNA according to claim 1 wherein said retinoic acid receptor is human retinoic acid receptor.
- [3. Substantially pure DNA according to claim 2 wherein said human retinoic acid receptor is selected from the group consisting of human retinoic acid receptor alpha and human retinoic acid receptor beta.]
4. Substantially pure DNA encoding protein which has [hormone-binding] hormone-binding and/or transcription-activating properties characteristic of retinoic acid receptor wherein said retinoic acid receptor is structurally and functionally related to the steroid and thyroid hormone receptors.
5. Substantially pure DNA according to claim 4 wherein said protein is human retinoic acid receptor.
- [6. Substantially pure DNA according to claim 5 wherein said human retinoic acid receptor is selected from the group consisting of human retinoic acid receptor alpha and human retinoic acid receptor beta.]
7. Substantially pure DNA sequences selected from the group consisting of DNA sequences shown in FIGS. 1B-1, 1B-2 and 1B-3.
8. DNA encoding chimeric receptors selected from the group consisting of GRR, GRG, GGR, RGG,

RGR, RRG, TTG, GTT, GTG, GGT, TGG, TGT, TTR, TRT, TRR, RTT, RTR, RRT, GTT, GTG, GGT, TGG, TGT, TTR, TRT, TRR, RTT, RTR, RRT, GTT, GTG, GGT, TGG, TGT, AND TTG.

9. Substantially pure DNA able to hybridize to the complementary strand of any of the [DNA's] DNAs claimed in any of claims [1-8] 1, 2, 4, 5 and 7, wherein said hybridizing DNA encodes retinoic acid receptor protein.

10. The plasmid phRAR1 (ATCC No. 40,392). 10

11. Cells transformed by any of the substantially pure [DNA's] DNAs claimed in any of claims 10 or [1-8] 1, 2, 4, 5 and 7 or any DNA able to hybridize to the complementary strand of said [DNA's] DNAs wherein said hybridizing DNA encodes retinoic acid receptor protein. 15

12. Cells transformed by any of the substantially pure DNAs [DNA's] claimed in any of claims 10 or [1-8] 1, 2, 4, 5 and 7, or any DNA able to hybridize to the complementary strand of said DNAs [DNA's], wherein said hybridizing DNA encodes retinoic acid receptor protein, and wherein said cells contain greater than wild-type amounts of retinoic acid receptor protein. 20

13. A method for producing retinoic acid receptor protein wherein said retinoic acid receptor protein is structurally and functionally related to the steroid and thyroid hormone receptors, said method comprising: 25

- a. ligating substantially pure DNA according to any of claims 10 or [1-8] 1, 2, 4, 5 and 7, or any DNA able to hybridize to the complementary strand of said DNAs [DNA's], wherein said hybridizing DNA encodes retinoic acid receptor protein, to a replicable expression vehicle to obtain a replicable recombinant DNA comprising said DNA and said replicable expression vehicle; 30
- b. transforming cells of a microorganism or cell culture with said replicable recombinant DNA to form transformants; 35
- c. selecting said transformants from patent cells of said microorganism or said cell culture; 40
- d. causing said transformants to express said DNA; and
- e. isolating retinoic acid receptor protein from said transformants. 45

14. A replicable recombinant DNA which comprises; a replicable vector; and substantially pure DNA according to any of claims 10 or [1-8] 1, 2, 4, 5 and 7 or any DNA able to hybridize to the complementary strand of said DNAs [DNA's], wherein said hybridizing DNA encodes retinoic acid receptor protein.

15. Substantially pure DNA able to hybridize to any of the DNAs claimed in any of claims 1, 2, 4, 5 and 7, wherein the complementary strand of said hybridizing DNA encodes retinoic acid receptor protein.